Abstract

Even though polyomaviruses have been intensively studied for more than 60 years, the role of minor structural proteins VP2 and VP3 in some important steps of viral life cycle has still not been fully elucidated, explicitly their role in viral genome delivery to the cell nucleus and their involvement in late phases of viral life cycle. This diploma thesis focuses on the study of minor proteins of Mouse polyomavirus (MPyV) and Human polyomavirus BK (BKV). Four rabbit polyclonal antibodies against minor proteins of polyomaviruses MPyV or BKV have been prepared within this diploma thesis. Two of these prepared antibodies target minor proteins of MPyV (α-MPyV VP2/3) or BKV virus (α-BKV VP2/3), other two prepared antibodies recognize C-terminal sequence common to minor proteins VP2 and VP3 of MPyV (α-MPyV C-termVP2/3) or BKV virus (α-BKV C-termVP2/3). In the second part of this diploma thesis we aimed to study toxicity of BKV virus minor proteins during individual production in mammalian cells. Obtained results suggest that minor proteins of BKV virus might not exhibit as high levels of cytotoxicity as minor proteins of MPyV virus. Third part of this diploma thesis is devoted to investigation of interactions of BKV and MPyV minor proteins with cellular proteins and within one another respectively. Macromolecular complexes of minor proteins were detected in lysates of mammalian cells transiently expressing minor proteins of MPyV or BKV viruses using prepared antibodies. We attempted to further characterize these complexes with the use of Blue-Native Protein Electrophoresis. Obtained results suggest that one of the identified complexes could contain both, minor proteins of BKV virus and cellular chaperon Hsp 70. Further analysis of these complexes was though not possible due to low proportion of complexes in cell lysate samples. Therefore, vectors for expression of MPyV or BKV minor proteins with C-terminal fusion tag capTEV were prepared. During production of fusion proteins in mammalian cells the capTEV tag is naturally biotinylated *in vivo* and enables isolation of fusion proteins by tandem affinity purification system. Although prepared vectors contain sequences for stabilization of mRNA transcripts from inserted gene, production of fusion minor proteins of MPyV nor BKV was detectable in mammalian cells. These vectors will though have to be adjusted for further use. Our future plan is to characterize interactions of both polyomaviral minor proteins in complexes formed during co-expression of minor protein and major capsid protein VP1 fused with capTEV tag. In this manner the cellular proteins interacting with the elementary building unit of viral capsid - whole complex of VP1 pentamer and minor protein could be revealed.

Key words: polyomavirus, Mouse polyomavirus, BK virus, minor structural proteins, VP2, VP3